Serine Protease Inhibitors

The present invention relates to serine protease inhibitors, cDNA coding for serine protease inhibitors, medicaments containing such inhibitors or their coding nucleic acid, use of the compounds according to the invention for the preparation of medicaments for the treatment of various indications, antibodies or antibody fragments against epitopes of the compounds according to the invention, poly- or oligonucleotides which will hybridize to genes of the compounds according to the invention, a diagnostic agent for detecting the compounds according to the invention, and medicaments containing antibodies or poly- or oligonucleotides according to the invention.

Proteolytic processes play an important physiological role in all organisms; a distinction has to be made between non-specific and specific proteolytic reactions. The former include, for example, the digestion of food in the digestive tract by endopeptidases, and the intracellular degradation of used endogenous substances and phagocytosed materials by lysosomal proteases. Specific proteolyses mostly serve for the conversion of a proenzyme to its active form, as in the conversion of trypsinogen to trypsin, and of chymotrypsinogen to chymotrypsin, and in the callicrein-kinin cascades and the blood clotting cascade. Depending on the structure of the reactive site of the proteinases involved, they are classified into the classes of serine proteases (e.g., chymotrypsin, trypsin, elastase and cathepsin G), aspartate proteases (e.g., cathepsin D, cathepsin E and pepsin), cysteine proteases (e.g., cathepsin B, cathepsin H and cathepsin L), and the metallo-proteases (e.g., collagenase and thermolysin).

In order to be able to correct the proteolytic processes which often proceed in a cascade, the organisms is provided with a number of other proteins, the protease inhibitors (for a survey, see Laskowski and Kato, 1980, and Bode and

Huber, 1992). Thus, the liver-synthesized human plasma protease inhibitors α_1-antichymotrypsin and α_1-proteinase inhibitors protect the lung tissue from non-specific attack by the proteinases cathepsin G and elastase from polymorphonuclear lymphocytes. When the balance between proteases and their specific inhibitors is disturbed, pathological effects may arise. For example, an excess ratio of elastase to α_1-proteinase inhibitor increases the risk of formation of a lung emphysema by a factor of about 20 to 30 in patients with a genetically caused deficiency in this factor as compared to the normal population (Carrel and Owen, 1980). With smokers, the formation of an emphysema is promoted by oxidation of the amino acid methionine which is present in the reactive site of the α_1-proteinase inhibitor by oxidants contained in cigarette smoke (Miller and Kuschner, 1969; Ohlsson et al., 1980). Also in the case of infection with Gram-negative bacteria, their endotoxins can cause disintegration of phagocytes and thus the secretion of lysosomal proteases, which may cause an uncontrolled damage to tissues and inflammations due to the increased consumption of protease inhibitors. For this reason, certain protease inhibitors have a high therapeutic potential (see, e.g., Fritz, 1980).

International Application PCT/EP 98/08424 relates to serine protease inhibitors, wherein said serine protease inhibitors have a domain with four cysteines, and a sequence of from 0 to 20 amino acids is present between the first and second cysteines, or said serine protease inhibitors have a domain of six cysteines, and a sequence of from 7 to 20 amino acids is present between the first and second cysteines.

It has been the object of the present invention to provide further serine protease inhibitors.

This object is achieved by a serine protease inhibitor having the amino acid sequence according to SEQ ID NO: 1.

The present invention also relates to fragments of the serine protease inhibitor according to the invention having the amino acid sequence R_1-X-R_2, wherein R_1 is NH_2, an amino acid or a peptide with up to 100 amino acids, and R_2 is COOH, $CONH_2$, an amino acid or a peptide with up to 100 amino acids, and X is selected from SEQ ID NOS: 2 to 6.

It is preferred that the serine protease inhibitor contains one or more disulfide bridges. It is particularly preferred for it to contain a disulfide bridge between the first and fourth cysteines and/or between the second and third cysteines, or to contain a disulfide bridge between the first and fifth cysteines and/or between the second and fourth cysteines and/or between the third and sixth cysteines.

In addition to the amino acid sequence of the preferred compounds according to the invention, further information about the cDNA coding for the compounds according to the invention can also be seen from Figure 1. In particular, the corresponding motifs and primer-hybridizing sites are indicated.

According to the invention, nucleic acids coding for the compounds according to the invention, especially a DNA having the nucleic acid sequence according to SEQ ID NOS: 7 to 12, are also claimed.

The compounds according to the invention are useful as medicaments. In this case, they are administered together with pharmaceutically acceptable vehicles.

The medicaments according to the invention containing the protease inhibitors according to the invention are preferably administered in amounts of from 1 to 100 mg/kg of the patient's body weight. As the dosage form, all galenic formulations for peptide active substances may be used. The medicaments containing nucleic acids according to the invention are preferably administered in amounts of from 0.1 to 100 mg/kg of body weight of a corresponding patient. In this case, the galenic dosage forms which may be used are those which are suitable for the administration of nucleic acids without rendering the nucleic

acids ineffective by metabolic influences before they have reached their site of action. For example, liposomes in which the nucleic acids are contained can be employed as a galenic dosage form.

The compounds according to the invention can be used, in particular, for the treatment of acute or chronic cervix inflammations, inflammations of Bartholin's gland or other vaginal regions, tonsillitis, pharyngitis and laryngitis, acute or chronic inflammatory processes accompanied by excessive formation of mucus and the resulting acute emergency situations, postoperative bleedings due to hyperfibrinolysis, and for the prophylaxis of lung emphysema formation in deficiencies of α_1-proteinase inhibitor.

Further, they may be employed for the therapy of asthma, AIDS, tumor diseases and leukemia.

The compounds according to the invention can be administered in deficiencies of serine protease inhibitors to correct endogenous defects. The nucleic acids may also be used in gene therapy, either directly or coupled to suitable vehicles. Suitable vectors include, in particular, attenuated adenoviruses into which the corresponding genes have been incorporated.

The polypeptides according to the invention can serve for the preparation of antibodies or antibody fragments. These are simply prepared by the immunization of appropriate mammals. By per se known operations, the antibodies may also be humanized so that such antibodies can also be employed for therapeutic use. Antibodies or antibody fragments can then by employed for the regulation of diseases in which the protease inhibitors are expressed in a pathological way. Also, antisense nucleic acids complementary to the nucleic acids according to the invention may be employed in therapeutical use in overexpressions of the protease inhibitor genes.

The compounds according to the invention can be easily prepared by per se known methods of peptide or nucleotide synthesis. Preparation of the compounds by genetic engineering is also possible.

Those skilled in the art will recognize that fragments of the polypeptides according to the invention may also be used provided that they retain the inhibitory properties of the serine protease inhibitors. Those skilled in the art know how to find such fragments. Thus, this may be accomplished, for example, by a selected enzymatic cleavage of the compounds according to the invention. Side-chain modified amino acids may also be employed. N- or C-terminally modified polypeptides may also be used. In particular, phosphorylated, glycosylated, methylated, acetylated or similarly modified polypeptides can be employed provided that they do not substantially affect the activity of the serine protease inhibitors.

Derivatives of the nucleic acids according to the invention which have modified triplet structures in accordance with codon usage may also be used. In addition, nucleic acids according to the invention also include those which are more stable towards degradation by nucleases as compared with the native compounds, for example, the corresponding SODN derivatives usually employed in antisense technology to give the antisense structures a more stable design towards enzymatic attack.

Structures homologous to the polypeptides may also be used. In particular, these include polypeptide structures in which amino acids have been exchanged. Thus, for example, conservative amino acid substitutions in highly conserved regions can be considered as follows: any isoleucine, valine and leucine amino acid can be exchanged for any other of these amino acids, aspartate can be exchanged for glutamate and vice versa, glutamine for asparagine and vice versa, serine for threonine and vice versa. Conservative amino acid substitutions in less highly conserved regions can be as follows: Any of the amino acids isoleucine, valine and leucine for any other of these amino acids, aspartate for

glutamate and vice versa, glutamine for asparagine and vice versa, serine for threonine and vice versa, glycine for alanine and vice versa, alanine for valine and vice versa, any of the amino acids leucine, isoleucine or valine for methionine, lysine for arginine and vice versa, either of the amino acids arginine or lysine for either of the amino acids aspartate or glutamate, either of the amino acids arginine or lysine for histidine, glutamine for glutamate and vice versa, and asparagine for aspartate and vice versa.